

- a) obtaining a fluorescently labeled oligonucleotide containing a nucleic acid sequence recognizable by an enzyme that facilitates nucleic acid ligation or cleavage;
- b) contacting said oligonucleotide with said enzyme that facilitates nucleic acid ligation or cleavage of said oligonucleotide; and
- c) continuously detecting said ligation or cleavage reaction by detecting a change in fluorescence intensity as said nucleic acid ligation or cleavage b) proceeds.

*Amended.*

16. The method of Claim 15, which is used to continuously detect a nucleic acid cleavage reaction by detecting an increase in fluorescence intensity.

17. The method of Claim 15, which is used to continuously detect a nucleic acid ligation reaction by detecting a decrease in fluorescence intensity.

18. The method of Claim 15, wherein cleavage is detected by measuring the change in fluorescence intensity by the equation:

$$[\text{DNA}]_c = \frac{F_+ - F_0}{F_\infty - F_0} \times [\text{DNA}]_i,$$

wherein  $[\text{DNA}]_c$  is the concentration of cleaved DNA,  $F_\infty$  is the fluorescence intensity at the plateau,  $F_0$  is the initial fluorescence intensity, and  $[\text{DNA}]_i$  is the initial concentration of DNA.

*Amended.* 19. The method of Claim 15, which is used to continuously detect said cleavage or ligation reaction while said cleavage or ligation reaction proceeds, in order to determine the kinetic profile of the reaction.

20. The method of Claim 15, wherein said oligonucleotide is fluorescently labeled at one end.

21. The method of Claim 20, wherein said fluorescent label is selected from the group consisting of fluorescein isothiocyanate, fluorescein amine, eosin, rhodamine, dansyl and umbelliferone.

22. The method of Claim 20, wherein said fluorescent label is quenched upon annealing of said oligonucleotide to its complementary strand.

23. The method of Claim 15, wherein said oligonucleotide is labeled with a fluorescence acceptor and fluorescence donor.

24. The method of Claim 23, wherein said fluorescent donor and acceptor respectively are fluorescein and eosin.

*Accepted.* 25. The method of Claim 23, wherein said fluorescent donor and acceptor respectively are fluorescein and tetramethyl rhodamine or fluorescein and tetramethyl rhodamine isothiocyanate.

26. The method of Claim 15, wherein the DNA or RNA is attached to a single fluorescent label.

27. The method of Claim 23, wherein the fluorescence donor is quenched when in close proximity of the fluorescence acceptor.

28. The method of Claim 23, wherein said oligonucleotide is fluorescently labeled at both ends.

29. The method of Claim 23, wherein said oligonucleotide is fluorescently labeled internally.

30. The method of Claim 23, wherein said fluorescent acceptor and donor are on the same strand of said oligonucleotide.

31. The method of Claim 23, wherein said fluorescent acceptor and donor are on different strands of said oligonucleotide.

*Amid* 32. The method of Claim 15, wherein said nucleic acid comprises DNA.

33. The method of Claim 15, wherein said nucleic acid comprises RNA.

34. The method of Claim 15, wherein said enzyme that facilitates said nucleic acid cleavage is a restriction enzyme.

35. The method of Claim 15, wherein said enzyme that facilitates said nucleic acid cleavage or ligation reaction is selected from the group consisting of a DNase, RNase, helicase, exonuclease, restriction endonuclease, and an integrase .

36. The method of Claim 15, wherein said enzyme that facilitates said nucleic acid cleavage or ligation reaction is a retroviral integrase enzyme.

37. The method of Claim 15, wherein said enzyme that facilitates said nucleic acid cleavage or ligation reaction is a polymerase.

*Accounted* 38. The method of Claim 15, wherein said enzyme that facilitates nucleic acid cleavage or ligation reaction is Rnase H.

39. The method of Claim 20, wherein said enzyme that facilitates said nucleic acid cleavage or ligation reaction is a restriction enzyme.

40. The method of Claim 23, wherein said enzyme that facilitates said nucleic acid cleavage or ligation reaction is a retroviral integrase enzyme.

41. The method of Claim 39, wherein said restriction enzyme is Bam H1.
42. The method of Claim 40, wherein said retroviral integrase enzyme is HIV integrase.
43. The method of Claim 15, wherein said enzyme mediated nucleic acid cleavage reaction occurs during a process for amplifying a specific DNA or RNA sequence.
- Accepted* 44. The method of Claim 43, wherein said process for amplifying or detecting a DNA or RNA sequence is catalytic hybridization amplification.
45. The method of Claim 43, wherein said process of amplifying a specific DNA or RNA sequence is a polymerase chain reaction.
46. The method of Claim 43, wherein said process of amplifying a specific DNA or RNA sequence is a ligase chain reaction.

47. The method of Claim 43, wherein said amplification is catalyzed by a polymerase.

48. The method of Claim 43, wherein said amplification process is catalyzed by Rnase H.

49. The method of Claim 43, wherein said enzyme that facilitates cleavage is a restriction endonuclease.

*Accepted.*  
50. The method of Claim 43, wherein said amplification is catalyzed by a ligase.

51. The method of Claim 15, wherein said enzyme mediated reaction is monitored continuously using a fluorescence microplate reader.

52. The method of Claim 43, wherein said enzyme mediated reaction is monitored continuously using a fluorescence microplate reader.

53. The method of Claim 43, wherein the amplified specific DNA or RNA sequence is detected continuously during the amplification reaction.

54. The method of Claim 53, which is used to quantitate the amount of an amplified DNA or RNA sequence over the entire amplification process.

55. The method of Claim 43, wherein said amplification process includes multiple cycles.

*accid.*  
56. The method of Claim 15, wherein said change in fluorescence is detected by single photon counting.

57. The method of Claim 15, wherein said change in fluorescence is detected by an analog method.

58. The method of Claim 15, wherein said change in fluorescence is measured by fluorescent lifetime.



59. The method of Claim 23, wherein said acceptor and donor are spaced within about zero to 20 bases of one another on said oligonucleotide.

60. The method of Claim 59, wherein said spacing ranges from zero to seven bases.

*Accepted.* 61. The method of Claim 15, wherein the sequence recognizable by a cleavage enzyme is about 6-7 nucleotides from the fluorescent label on the oligonucleotide.

62. The method of Claim 15, wherein the fluorescent label is attached to the oligonucleotide by a linker.

63. The method of Claim 62, wherein said linker is a carbon linker comprising a 12 carbon chain.--